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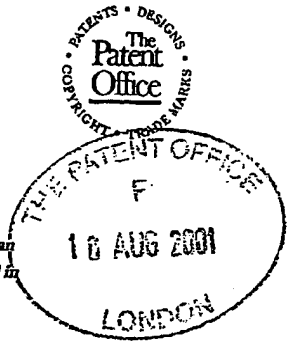

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Medical Research Council
20 Park Crescent
London
W1N 4AL

Patents ADP number *(if you know it)*

5840602400 1

If the applicant is a corporate body, give the country/state of its incorporation

United Kingdom

4. Title of the invention

Molecule

5. Name of your agent *(if you have one)*

D Young & Co

"Address for service" in the United Kingdom to which all correspondence should be sent *(including the postcode)*

21 New Fetter Lane
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Signature D Young & Co Date 10 August 2001

D Young & Co (Agents for the Applicants)

12. Name and daytime telephone number of person to contact in the United Kingdom — Chong-Yee Khoo — 023 8071 9500

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MOLECULE

FIELD

The present invention relates to polypeptides, and in particular molecules capable of stabilising native conformations of a polypeptide.

BACKGROUND TO THE INVENTION

The maintenance of a tertiary structure is crucial for protein activity. Thus, the conformation of a plays an essential part in its ability to bind another molecule, or for its enzymatic activity. When protein conformation is disrupted, for example, by denaturation, activity may be lost.

The tumour suppressor protein p53 plays a key role in the protection of cells from cancer. It is a transcription factor, which exists in low levels in normal cells and is induced in response to DNA damage or to other conditions under which there is a danger to normal cell growth (reviewed in Hupp *et al.*, 2000; Sigal and Rotter, 2000). Following the increase in its cellular level, p53 activates several genes, and triggers cellular processes that prevent the proliferation of the genetically impaired cells. This is achieved by mediating cell-cycle arrest or by apoptosis.

More than 50% of human cancers have mis-sense mutations in the gene coding for p53 that result in its inactivation (Hainaut and Hollstein, 2000). Nearly all such mutations are in the DNA-binding core domain (Hainaut and Hollstein, 2000). The six most frequent cancer-associated mutations are the "hot-spots" R175H, G245S, R248Q, R249S, R273H and R282W. Based on the crystal structure of p53 core domain (Cho *et al.*, 1994), these mutations can be divided into two categories: (1) DNA-contact mutations (R248 and R273), which result in loss of DNA-binding residues, and (2) "structural mutations", which result in structural changes in p53 core domain that can range from local distortion to complete unfolding. A new assessment of the mutation database (Bullock *et al.*, 2000), based on thermodynamic stability and DNA binding properties of the mutants, classifies

three broad phenotypes: (I) DNA-contact mutations that have little effect on folding/stability (e.g. R273H) (ii) mutations that cause a local distortion, mainly in proximity to the DNA binding site (e.g. R249S, which are usually destabilised by <2 kcal/mol); and (iii) mutations that cause global unfolding (e.g. mutations in the core domain sandwich) that are destabilised by >3 kcal/mol.

Activation of mutant core domain by short peptides derived from the regulatory C-terminal domain of p53 (Abarzua *et al.*, 1996; Hupp *et al.*, 1995; Selivanova *et al.*, 1997; Selivanova *et al.*, 1999) has been proposed as a means to stabilise p53. These peptides work by specifically regulating the core domain activity rather than stabilising it. Accordingly, such prior polypeptides are not relevant to the invention disclosed here.

It is a problem in the art to provide a means to rescue p53 mutants, and other mutants in tumour suppressor proteins, to restore tumour suppression activity for cancer therapy. Mutations in oncogenes are also known to cause tumour activity. It is a further problem in the art to provide means to rescue such oncogenic mutations.

SUMMARY

We have realised for the first time that different classes of mutants of tumour suppressor proteins and oncogene proteins require different rescue strategies. In order to rescue DNA contact mutants of tumour suppressor proteins, for example, there is a need to introduce functional groups that will establish new contacts with the DNA, compensating for the missing contacts. We have discovered that rescue of globally unfolded or locally distorted mutants may be achieved by stabilisation that will lead to refolding of the mutant, which in turn will lead to restoration of the wild-type p53 activity.

It has been reported that the rescue of mutant p53 may be achieved by small molecules, e.g. CP-31398. CP-31398 is said to stabilise only newly synthesised p53 that is in the active conformation, which then allows the time dependent accumulation of this

fraction (Foster *et al.*, Science, vol 286, 1999, 2507-2510). However, we and others have not found that CP-31398 does not in fact work to stabilise active conformations of p53.

We therefore provide for the first time a molecule which is capable of binding a native conformation of a protein, such that the binding stabilises the native conformation. We term such a molecule a "stabilising molecule". Stabilisation of the native conformation enables the equilibrium between an unfolded, denatured and/or inactive conformation of the polypeptide and a properly folded, native and active form to shift towards the latter. Accumulation of native protein therefore results.

There is provided, according to a second aspect of the present invention, a method of increasing the concentration of a native state of a reversibly denatured polypeptide in a system, in which the system comprises the polypeptide in a first native state and a second denatured state, the method comprising: (a) providing a stabilising molecule which binds to the polypeptide at a site which at least partially overlaps a functional site in the first native state and thereby stabilising the first native state of the polypeptide; and (b) allowing the stabilising molecule to bind to the polypeptide.

We provide, according to a third aspect of the present invention, a method of restoring a wild type phenotype of an organism comprising a mutation in a polypeptide, in which the mutation leads to denaturation of the polypeptide and a mutant phenotype, the method comprising exposing the organism or part of the organism to a stabilising molecule which binds to the polypeptide at a site which at least partially overlaps a functional site in its native state and thereby stabilises the native state of the polypeptide.

As a fourth aspect of the present invention, there is provided a method of treatment of a disease in a patient, in which the disease is caused by or associated with a mutation in a polypeptide which leads to denaturation of the polypeptide, the method comprising administering to the patient a stabilising molecule which binds to the polypeptide at a site which at least partially overlaps a functional site in its native state and thereby stabilises the native state of the polypeptide.

In a preferred embodiment, the stabilising molecule is not a natural binding partner of the polypeptide. Preferably, the stabilising molecule consists of a fragment of a natural binding partner of the polypeptide. More preferably, the stabilising molecule is a polypeptide engineered to include a polypeptide binding domain, preferably a binding loop, of a natural binding partner of the polypeptide.

The stabilising molecule may be exposed to polypeptide or the system in presence of a natural binding partner of the polypeptide. Preferably, the affinity of binding between stabilising molecule and the polypeptide or binding site is less than the affinity of a natural binding partner of the polypeptide and the polypeptide or the binding site. More preferably, binding between the stabilising molecule and the binding site stabilises the polypeptide to enable binding between the polypeptide and a natural binding partner. Most preferably, binding between the polypeptide and the natural binding partner stabilises the native state of the polypeptide.

We provide, according to a fifth aspect of the present invention, a method of assisting the binding between a polypeptide and a natural binding partner for the polypeptide, the method comprising stabilising a native state of the polypeptide by a method according to any preceding claim, and exposing the stabilised polypeptide to the natural binding partner.

The present invention, in a sixth aspect, provides a method of assisting the binding between a polypeptide and a first molecule, in which the polypeptide exists in a native state and a denatured state, the method comprising: (a) providing a second stabilising molecule capable of binding to a site which at least partially overlaps a functional site in the native state of the polypeptide; (b) allowing the second stabilising molecule to bind to the polypeptide to form a complex and thereby stabilising the native state of the polypeptide; (c) exposing the polypeptide and bound second stabilising molecule complex to the first molecule; and (d) allowing the first molecule to bind to the polypeptide and thereby displacing the second stabilising molecule.

The functional site preferably comprises or at least partially overlaps with o a structural domain, a protein binding domain, a nucleic acid binding domain, or an active site of an enzyme. More preferably, the functional site is essential to the structure or activity, or both, of the polypeptide.

In a highly preferred embodiment of the invention, the polypeptide comprises an oncogenic protein or a tumour suppressor protein. Preferably, the polypeptide is p53. More preferably, the polypeptide is p53 which comprises a mutation, preferably R175H, G245S, R248Q, R249S, R273H and R282W, in which the mutation leads to reversible denaturation of the polypeptide.

The stabilising molecule may comprise a CDB3 polypeptide having the sequence REDEDEIEW.

In a seventh aspect of the present invention, there is provided a stabilising molecule which binds to and stabilises the native state of a polypeptide, but not a denatured state of the polypeptide, in which the stabilising molecule binds to a site which at least partially overlaps a functional site of the polypeptide, and in which the stabilising molecule does not consist of a natural binding partner of the polypeptide.

Preferably, the polypeptide is p53. More preferably, the polypeptide is p53 which comprises a mutation, preferably R175H, G245S, R248Q, R249S, R273H and R282W, in which the mutation leads to reversible denaturation of the polypeptide. Most preferably, the stabilising molecule comprises a CDB3 polypeptide having the sequence REDEDEIEW.

According to an eighth aspect of the present invention, we provide a method of identifying a stabilising molecule capable of stabilising a polypeptide, in which the polypeptide may be reversibly denatured such that it exists in a native state and a denatured state, the method comprising the steps of: (a) providing a native state of the polypeptide comprising a functional site; (b) exposing the polypeptide to a candidate

stabilising molecule; (c) selecting a candidate stabilising molecule which binds to a site which at least partially overlaps a functional site of the native state of the polypeptide; and (d) determining whether such binding stabilises the native state of the polypeptide.

We provide, according to a ninth aspect of the invention, a method of identifying a stabilising molecule capable of stabilising a polypeptide, in which the polypeptide may be reversibly denatured such that it exists in a native state and a denatured state, the method comprising the steps of: (a) identifying a functional site of the polypeptide and providing a polypeptide fragment comprising the functional site; (b) selecting a candidate stabilising molecule which binds to the polypeptide fragment at a site which at least partially overlaps a functional site; (c) determining whether the selected candidate stabilising molecule stabilises a native state of a polypeptide.

The polypeptide fragment may comprise the functional site includes a binding site for a natural binding partner of the polypeptide.

There is provided, in accordance with a tenth aspect of the present invention, a stabilising molecule capable of stabilising a polypeptide, which is identified by a method according to the previous two aspects of the invention.

A stabilising molecule as described here preferably comprises a natural or derivatised carbohydrate, protein, polypeptide, peptide, glycoprotein, nucleic acid, DNA, RNA, oligonucleotide or protein-nucleic acid (PNA). The methods as described here may employ such a derivatised or natural stabilising molecule. More preferably, the stabilising molecule is derivatised with a sugar, phosphate, amine, amide, sulphate, sulphide, biotin, a fluorophore or a chromophore. Most preferably, the stabilising molecule is derivatised using a fluorophore, preferably fluorescein.

The binding of a stabilising molecule to the polypeptide may be detected using NMR spectroscopy, preferably heteronuclear NMR spectroscopy, fluoresecence anisotropy, surface plasmon resonance, or Differential Scanning Calorimetry (DSC).

As an eleventh aspect of the invention, we provide a stabilising molecule according to the relevant previous aspects of the invention for use in the treatment of a disease.

We provide, according to a twelfth aspect of the invention, there is provided a pharmaceutical composition comprising a stabilising molecule as described here together with a pharmaceutically acceptable carrier, diluent or exipient.

According to a thirteenth aspect of the present invention, we provide use of stabilising molecule as described here in the manufacture of a medicament for treatment of a disease.

There is provided, according to a fourteenth aspect of the present invention, use of a stabilising molecule as described here in the treatment of disease. In a highly preferred embodiment of the invention, the disease is cancer.

BRIEF DESCRIPTION OF THE FIGURES

Figure 1 shows the crystal structure of the p53 core domain (blue)-53BP2 (red) complex (coordinates taken from Gorina and Pavletich, 1996) with the three 53BP2 derived peptides synthesized for this study highlighted : CDB1 (residues 422-428) - green, CDB2 (residues 469-477) – yellow, CDB3 (residues 490-498) – purple. Picture is generated using swissPDB viewer (Guex and Peitsch, 1997).

Figure 2 shows a 1H, 15N HSQC spectra of p53 core domain in the presence (red) and the absence (black) of CDB3. Selected residues that show significant chemical shift deviation in presence of CDB3 are highlighted.

Figure 3: shows the binding of p53 core domain to immobilised peptides analysed by surface plasmon resonance. (a) Screening for p53 core domain binding peptides. Biotinylated peptides are immobilised on a streptavidin BIAcore chip and p53 core domain (7.2 μM) is injected. The values shown are normalised by the response upon p53

injection to the flow channel without any immobilised peptide.

(b) Concentration dependence of p53 core domain binding to immobilised CDB3.

(c) Titration of CDB3 binding to p53 core domain by competition BIAcore. The concentration of free p53 core domain (reflected by association rate in binding to immobilized CDB3) is analyzed by BIAcore after incubation of 0.2μM p53 core domain and various concentrations of free CDB3.

Figure 4 shows the chemical shift changes (∂) in p53 core domain upon binding to CDB3. (a) ^{1}H and ^{15}N Chemical shift deviations plotted against residue number. Deviations above 5 times the standard deviation (∂>0.25 ppm for ^{15}N and ∂>0.05 ppm for ^{1}H) are considered significant (white background). ∂ differences between 2.5 times and 5 times the standard deviation (0.125< ∂<0.25 ppm for ^{15}N, 0.025< ∂<0.05 ppm for ^{1}H) are considered as minor (light grey background), and ∂ differences below 2.5 times the standard deviation (∂<0.125 ppm for ^{15}N and ∂<0.025 ppm for ^{1}H) are considered insignificant (dark grey background).

(b) Chemical shift changes in the p53 core domain structure upon CDB3 binding. Residues with significant chemical shift changes are coloured blue, residues with minor changes are coloured purple and residues with no change are coloured yellow. CDB3 in its original position in the 53BP2-p53 complex is shown in red (coordinates taken from (Gorina and Pavletich, 1996)).

Figure 5 shows the CDB3 binding to p53 core domain analysed by anisotropy and fluorescence. (a) Wild-type and mutant p53 core domain are titrated into a fluorescein-labeled CDB3 (4.6 μM). Changes in anisotropy are monitored and analysed. (b) Competition experiment where unlabeled or biotinylated CDB3 are titrated into 0.50 μM fluorescein-labeled CDB3 and 2.0 μM p53 core domain wild-type (■ and , 0.2□mM and 2.6 mM unlabeled CDB3, respectively, and ●, 0.24 mM biotinylated CDB3).

Figure 6 shows the stabilisation of p53 core domain by FL-CDB3. (a) differential scanning calorimetry. The apparent T_m of wild-type and R249S core domain in the presence or absence of FL-CDB3 is determined as described in materials and methods. For

the wild-type core domain T_m=40.1 °C in the absence of the peptide and 41.6 °C in its presence. For R249S T_m=34.9 °C in the absence of the peptide and 35.9 °C in its presence. Raw data are shown and are offset for clarity. (b-c) Urea dependence of p53-CDB3 binding. Wild-type p53 core domain is titrated into fluorescein-labeled CDB3 in presence of increasing urea concentrations, and changes in anisotropy are monitored. (b) anisotropy titration curves under various urea concentrations (c) log K_d for the p53 core domain-CDB3 interaction versus urea concentration (d) CDB3 induces refolding of p53 core domain. Wild-type p53 core domain is pre-incubated overnight with 3 M urea, then mixed with fluorescein-labeled CDB3 and the anisotropy change over time is monitored. As a control, the same protein is mixed with 3M urea and with fluorescein-labeled CDB3 without pre-incubation and anisotropy changes over time are monitored.

Figure 7 shows the "Chaperone" strategy for rescue of p53. (a) DNA competes with FL-CDB3 on p53 core domain binding. 30-mer gadd-45 DNA (+ = 25μM, ■ =5μM) is titrated into a mixture of p53 core domain-FL-CDB3 as described in materials and methods. (b) The chemical shift changes (∂) in p53 core domain upon binding to 12-mer DNA. Residues that are shifted so far that they could not be re-assigned are coloured red, residues with significant chemical shift changes (∂>0.25 ppm for ^{15}N and ∂>0.05 ppm for ^{1}H and see legend for figure 4) are coloured blue, and residues with no change are coloured yellow (coordinates taken from (Gorina and Pavletich, 1996)). Picture is generated using swissPDB viewer. (c) A schematic model of the proposed mechanism of action for CDB3. See text for details.

DETAILED DESCRIPTION OF THE INVENTION

The invention relies on the provision of a stabilising molecule which is capable of binding to a native form of a polypeptide, thereby stabilising it.

Where the polypeptide exists in equilibrium between a native, properly folded or active form and a denatured, unfolded or inactive form, binding of the stabilising molecule to the native form of the polypeptide stabilises it and drives the equilibrium towards the

folded, active or native form. Thus, the stabilising molecule is capable of increasing the relative concentration of a native form of a polypeptide as compared to a denatured form. Such a stabilising molecule will bind the native, but not the denatured state of the polypeptide. The law of mass dictates that in such a case the equilibrium will be shifted towards the native state and the amount of active protein will increase.

Preferably, the polypeptide is reversibly denatured. In other words, a proportion of the polypeptide molecules in any system is in the native, folded, or active form, and a proportion of the polypeptide molecules is in the inactive, unfolded (whether partially or fully) or denatured form. Such denaturation may arise though various means, and the invention is suitable for use in any of these situations. Thus, the polypeptide may be exposed to an environment which results in its denaturation; for example, by being exposed to a non-physiological environment. The polypeptide may be oxidised by exposure to air, or denatured by exposure to heat, high or low salt concentrations, etc. The polypeptide may be denatured by virtue of a co-factor being removed from it.

In a highly preferred embodiment, however, the reversible denaturation of the polypeptide results from genetic mutation. Thus, a mutation in the sequence of the polypeptide results in its destabilisation and tendency to denature. Preferably, such a mutation results in loss of activity of the polypeptide. The mutation may result in a mutant phenotype of the polypeptide, or cell, tissue or organism comprising the mutant phenotype, such a mutant phenotype being different in some detectable way from a wild type phenotype associated with a unmutatated or wild type polypeptide. The methods of our invention are therefore suitable for rescuing such a mutant phenotype. These methods may also be used to rescue a mutant form of a protein, for example, an oncogene protein or a tumour suppressor protein, by a stabilising molecule binding to the native state of the protein, but not the denatured state, and thereby shifting the equilibruim which exists between the two forms to the native state.

It is known that mutated forms of oncogenes and tumour suppressor proteins are involved in tumourogenesis. As noted above, such mutations may lead to partial

denaturation of the polypeptide and loss of activity. Therefore the methods of our invention are suitable for stabilising such mutated oncogenes and/or tumour suppressor proteins and restoring wild type activity. Accordingly, the methods described here are suitable for rescuing wild type activity of oncogenes and tumour suppressors, and hence of preventing tumourogenesis and/or cancer. Preferably, the oncogene comprises p21ras, or any other oncogene known in the art. Preferably, the tumour suppressor comprises p53 or retinoblastoma protein. The p53 may comprise a mutation leading to partial denaturation, preferably reversible denaturation. Examples of such mutations include R175H, G245S, R248Q, R249S, R273H and R282W..

Furthermore, it is known that many diseases are caused by or associated with polypeptide mutations, which mutations may lead to destabilisation and reversible denaturation of the protein. Administration of a stabilising molecule as described here to a patient suffering from such a disease will stabilise the native form of the polypeptide, and increase the amount or relative concentration of the native form over the denatured form. Accordingly, administration of stabilising molecules may be used to treat diseases associated with or caused by such mutations.

In a highly preferred embodiment, the stabilising molecule binds to a site which comprises or at least partially overlaps a functional site in the polypeptide. Preferably, the site at which the stabilising molecule binds overlaps or consists of the functional site. Such a functional site preferably comprises a site which is essential for a relevant activity of the polypeptide. The functional site may also be essential for the structure of the polypeptide. The functional site may be an interaction site, which interacts with another molecule in the cell, such as a natural binding partner of the polypeptide including another polypeptide, a small molecule, a ligand, a macromolecule, a nucleic acid, etc.

Examples of such functional sites include active sites, or substrate binding sites, where the polypeptide is an enzyme. In the case of binding proteins, the functional site comprises, or at least overlaps, a binding site or binding domain of the polypeptide. Thus, in the case of nucleic acid binding sites, the functional site comprises a nucleic acid

binding site, such as a DNA binding site in a DNA binding protein, or an RNA binding site in a RNA binding protein. Where the polypeptide interacts with another polypeptide, i.e., has polypeptide binding activity, the functional site preferably comprises a polypeptide interaction domain or sequence, i.e., it includes, overlaps, or is a sequence which interacts with another polypeptide.

Preferably, therefore the stabilisation of the native state of the polypeptide enables the binding of another molecule to the polypeptide. This other molecule is preferably a different molecule or unrelated molecule to the stabilising molecule. Thus, stabilisation of the polypeptide by the stabilising molecule preferably enables a proper conformation of the functional site to be maintained in the polypeptide, to allow the binding of the other molecule. Preferably, the other molecule is a natural binding partner of the polypeptide, for example, a DNA where the polypeptide is a DNA binding protein.

Thus, the stabilising molecule is capable of competing with the binding of a natural binding partner of the polypeptide for binding to the polypeptide or the functional site. Preferably, however, the affinity of binding of the stabilising molecule to the polypeptide is less than the affinity of binding of a natural binding partner to the polypeptide. Thus, the natural binding partner is capable of displacing the stabilising molecule from the functional site, or the binding site of the natural binding partner. Thus, in this preferred embodiment, the binding of the stabilising molecule to the polypeptide stabilises the native state of the polypeptide for long enough to enable binding of the natural binding partner to the polypeptide.

Binding of the stabilising molecule to the native state shifts the equilibrium to this state. Preferably, therefore, the stabilising molecule does not require energy for its stabilising stabilising activity. The stabilising molecule as described here does not actively refold the polypeptide, in contrast to classic chaperone activity.

Preferably, the functional site exists only in the native, active or properly folded form of the polypeptide. More preferably, the functional site does not exist in the

denatured form of the polypeptide. Preferably, the affinity of binding of the stabilising molecule to the native form of the polypeptide is greater than the affinity of binding to the denatured form of the polypeptide. In a highly preferred embodiment, the stabilising molecule substantially only binds to the native form and not the denatured form of the polypeptide.

While small stabilising molecules are included, preferred stabilising molecules comprise polypeptides, preferably derived from natural binding partners of the polypeptide to be stabilised. This overcomes the difficulty and expense of synthesising small molecules. In addition, it is often difficult to scale up the synthesis procedure of identified small molecules.

Although natural binding partners of the polypeptide to be stabilised may be used as stabilising molecules, a highly preferred embodiment relies on the use of a stabilising molecule which is not a natural binding partner of the polypeptide. By this we mean that the stabilising molecule is preferably an engineered molecule, which does not exist in nature, but which is capable of binding to the polypeptide in its native form and stabilising it. Engineered stabilising molecules may be generated by means known in the art, including recombinant DNA technology. They preferably comprise or consist of fragments of natural binding partners, preferably fragments comprising binding activity. Thus, for example, where the stabilising molecule is a polypeptide, this suitably consists of or comprises a polypeptide binding sequence, loop or domain. An example of this is a stabilising molecule consisting of CDB3, which is a fragment of a p53 binding polypeptide 53BP2 (accession number NM_005426.1).

One skilled in the art will appreciate that the stabilising molecule may act in isolation in the rescue of mutant proteins. Alternatively, it may act in conjunction with another peptide, or other stabilising molecule in the rescue of the protein. There may be an additive effect between one or more peptides or molecules, alternatively they may act synergistically.

In a preferred embodiment, the polypeptide is an oncogenic protein or a tumour suppressor protein, preferably a mutant oncogenic protein or a mutant tumour suppressor protein. Advantageously, the protein is p53, preferably a mutant of p53. The tumour suppressor protein may comprise retinoblastoma protein (RB). Those skilled in the art will appreciate this list is by no means exhaustive.

The binding of the stabilising molecule to the native polypeptide may detected using any suitable means known in the art. Preferred means include physical methods such as NMR spectroscopy. In a preferred embodiment the NMR involves the use of heteronuclear NMR spectroscopy. The binding may also be detected using surface plasmon resonance. Alternatively, the binding of the stabilising molecule to the native form of the polypeptide is detected using Differential Scanning Calorimetry (DSC) and or fluorescence anisotropy. All of these methods will be familiar to those skilled in the art and are described in detail in this document.

In an alternative embodiment, the binding of the stabilising molecule to each state of the polypeptide, i.e., native or denatured, may be detected by examining the fraction of the polypeptide sample which expresses an epitope for one or more monoclonal antibodies, which epitopes are only present in one form of the polypeptide. Other suitable methods for detecting conformational changes in proteins include, but are not limited to electrophoresis and thin-layer chromatography. Those skilled in the art will be aware of other suitable methods.

In a particular embodiment, the polypeptide comprises a DNA binding protein. A mutated form of the DNA binding polypeptide comprises a denatured form which is incapable of binding DNA. A stabilising molecule is provided which binds an unfolded or distorted oncogenic protein which is unable to bind DNA, and shifts the equilibruim which exists between the denatured state and the native 'wild-type' state towards the latter. DNA can then bind the mutated protein, displacing the molecule, which is preferably a peptide, so that it is free again to bind another protein molecule.